**Figure 4. Sequence-specific Cre-mediated induction of RNAi in ES cells.** (A1)IRES-GFP transgenic U6lox-shA1 KI ES cells were transduced with Cre-expressing adenovirus and analyzed 7 days thereafter. **(a)** FACS analysis of GFP expression in transduced (open histograms) or untransduced ES cells (shaded histograms). The respective (A1)IRES-GFP transgene is indicated. AV, adenovirus. **(b)** PCR analysis to detect Cre-mediated deletion of the polIII STOP cassette. A schematic of the targeted HPRT locus is shown, half-arrows depict primers hHPRT-pro and HPRT-SAH (see Table I) flanking the inserted U6lox-shA1 cassette. The arrow represents the human HPRT promoter, the gray box depicts human exon 1, the white box mouse exon 2; map is not drawn to scale. PCR results are shown for transduced and untransduced ES cell transgenic for IRES-GFP (IRES), A1-IRES-GFP (A1) or mutA1-IRES-GFP (mutA1). A1-IRES-GFP transgenic ES cells were sorted according to GFP expression levels. DNA from $GFP^{high}$ cells and $GFP^{low}$ cells was subjected to PCR. The expected sizes for PCR fragments before (U6-STOP-A1) and after deletion of the polIII STOP cassette (U6-A1) are indicated. The asterisk indicates a fragment resulting from a DNA hybrid of one U6-STOP-A1 strand and one U6-A1 strand. **(c)** Northern blot analysis of siA1 expression in transduced and untransduced ES cells carrying the indicated transgene. 20 μg of total RNA were loaded per lane. Synthetic ds siRNA of identical sequence was loaded as indicated to estimate siRNA expression levels. **(d)** Northern blot analysis of (A1)IRES-GFP mRNA expression levels before and after transduction. 20 μg of total RNA were loaded per lane. Cre-transduced A1-IRES-GFP transgenic ES cells were sorted according to GFP expression levels and total RNA from $10^6$ cells was loaded for $GFP^{high}$ and $GFP^{low}$ samples. Targeted ES cells without IRES-GFP transgene served as negative control (–). Blots were hybridized to GFP probe. To account for loading differences, blots were stripped and rehybridized to GAPDH probe. The efficiency of siA1 mediated A1-IRES-GFP knock down was determined using phosphoimager.